

PB87-115093

Effects of Inhalation of Organic
Chemical Air Contaminants on
Murine Lung Host Defenses

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

1986



U.S. Department of Commerce
National Technical Information Service

NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA/600/J-86/177		3. F PB87-115093	
4. TITLE AND SUBTITLE The Effects of Inhalation of Organic Chemical Air Contaminants on Murine Lung Host Defenses (Journal Version)		5. REPORT DATE 1986	
7. AUTHOR(S) Aranyi*, O'Shea*, Graham†, Miller†		6. PERFORMING ORGANIZATION CODE	
9. PERFORMING ORGANIZATION NAME AND ADDRESS *IIT Research Institute, Chicago †EPA/Health Effects Research Laboratory RTP, NC		8. PERFORMING ORGANIZATION REPORT NO.	
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory - RTP, NC Office of Research and Development U.S. Environmental Protection Agency Research Triangle Park, NC 27711		10. PROGRAM ELEMENT NO.	
		11. CONTRACT/GRANT NO.	
		13. TYPE OF REPORT AND PERIOD COVERED	
		14. SPONSORING AGENCY CODE EPA/600/11	
15. SUPPLEMENTARY NOTES Fundamental and Applied Toxicology (6, 713-720 1986)			
16. ABSTRACT The Effects of Inhalation of Organic Chemical Air Contaminants on Murine Lung Host Defenses. ARANYI, C., O'SHEA, W. J., GRAHAM, J. A., AND MILLER, F. J. (1986). <i>Fundam. Appl. Toxicol.</i> 6, 713-720. The potential health hazards of exposure to threshold limit value (TLV) concentrations of acetaldehyde, acrolein, propylene oxide, chloroform, methyl chloroform, carbon tetrachloride, allyl chloride, methylene chloride, ethylene trichloride, perchloroethylene, benzene, phenol, monochlorobenzene, and benzyl chloride, compounds which may be present in the ambient or work room atmosphere were investigated. The effects of single and multiple 3-hr inhalation exposures were evaluated in mice by monitoring changes in their susceptibility to experimentally induced streptococcus aerosol infection and pulmonary bactericidal activity to inhaled <i>Klebsiella pneumoniae</i> . When significant changes in these parameters were found, further exposures were performed at reduced vapor concentrations until the no-measurable-effect level was reached. Multiple exposures on 5 consecutive days were then performed at this concentration. Significant increases in susceptibility to respiratory streptococcus infection were observed after single 3-hr exposure to TLV concentrations of methylene chloride, perchloroethylene, and ethylene trichloride. For methylene chloride and perchloroethylene, these exposure conditions also resulted in significantly decreased pulmonary bactericidal activity. © 1986 Society of Toxicology.			
17. KEY WORDS AND DOCUMENT ANALYSIS			
a. DESCRIPTORS		b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
REPRODUCED BY U.S. DEPARTMENT OF COMMERCE NATIONAL TECHNICAL INFORMATION SERVICE SPRINGFIELD, VA 22161			
18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC		19. SECURITY CLASS (This Report) UNCLASSIFIED	21. NO. OF PAGES 10
		20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE

The Effects of Inhalation of Organic Chemical Air Contaminants on Murine Lung Host Defenses¹

CATHERINE ARANYI,*² WILLIAM J. O'SHEA,* JUDITH A. GRAHAM,†
AND FREDERICK J. MILLER†

*IIT Research Institute, Life Sciences Research Department, 10 West 35th Street, Chicago, Illinois 60616; and
†Toxicology Branch, Health Effects Research Laboratory, U.S. EPA, Research Triangle Park, North Carolina 27711

The Effects of Inhalation of Organic Chemical Air Contaminants on Murine Lung Host Defenses. ARANYI, C., O'SHEA, W. J., GRAHAM, J. A., AND MILLER, F. J. (1986). *Fundam. Appl. Toxicol.* 6, 713-720. The potential health hazards of exposure to threshold limit value (TLV) concentrations of acetaldehyde, acrolein, propylene oxide, chloroform, methyl chloroform, carbon tetrachloride, allyl chloride, methylene chloride, ethylene trichloride, perchloroethylene, benzene, phenol, monochlorobenzene, and benzyl chloride, compounds which may be present in the ambient or work room atmosphere were investigated. The effects of single and multiple 3-hr inhalation exposures were evaluated in mice by monitoring changes in their susceptibility to experimentally induced streptococcus aerosol infection and pulmonary bactericidal activity to inhaled *Klebsiella pneumoniae*. When significant changes in these parameters were found, further exposures were performed at reduced vapor concentrations until the no-measurable-effect level was reached. Multiple exposures on 5 consecutive days were then performed at this concentration. Significant increases in susceptibility to respiratory streptococcus infection were observed after single 3-hr exposure to TLV concentrations of methylene chloride, perchloroethylene, and ethylene trichloride. For methylene chloride and perchloroethylene, these exposure conditions also resulted in significantly decreased pulmonary bactericidal activity. © 1986 Society of Toxicology.

Increasing attention is being directed toward identification and evaluation of toxic air pollutants found in the ambient air or in occupational settings. Hundreds of new chemicals are being introduced annually into the marketplace, many of which are of unknown inhalation hazard. The chemical compounds used in this study (Table I) were selected from a list of potential hazardous air contaminants undergoing risk assessment by the EPA. The 1981 production volumes in the U.S. for these various compounds ranged from approximately 50,000 to 5,000,000 tons (U.S. International Trade Comm., 1982). In order to better assess the risks of such chemicals and those already known to pose threats, more information is needed regarding their impact on

health. These studies examined the potential of a number of vapor-phase chemicals to alter host susceptibility to laboratory-induced respiratory infection in mice.

METHODS

Animals. Four-to-five-week old female CD1 mice³ housed in groups of six in plastic cages were quarantined for 7-14 days. Food and water were provided *ad libitum* except during exposures. During the exposures to organic vapors or to filtered air, and for the infectious and radioactive bacterial aerosol challenges, the mice were individually confined in stainless-steel wire cages within the inhalation chambers.

Test articles. Chloroform,⁴ carbon tetrachloride,⁴ methylene chloride,⁴ benzene,⁴ acrolein,⁵ allyl chloride,⁵

¹ Presented in part at the 23rd Annual Meeting of The Society of Toxicology, Atlanta, Ga., March 12-16, 1984.

² To whom correspondence should be addressed.

³ Charles River Breeding Laboratories, Portage, Mich.

⁴ B and J Laboratories, Muskegon, Mich.

⁵ Eastman Kodak Co., Rochester, N.Y.

and perchloroethylene⁵ were obtained as spectro-grade chemicals, whereas certified reagent grades of acetaldehyde,⁶ propylene oxide,⁶ methyl chloroform,⁶ ethylene trichloride,⁶ phenol,⁶ benzyl chloride,⁶ and monochlorobenzene⁶ were used.

Generation and monitoring the test atmosphere. Inhalation chambers were 342-liter glass aquaria fitted with glass covers. To assure homogeneous distribution, the test atmosphere was introduced and exhausted through areas separated by perforated stainless-steel diffusion plates at each end of the aquarium. Chamber intake and dilution air were provided by passing room air through a canister containing a coarse filter, a charcoal cartridge, and a HEPA filter. Chamber exhaust was passed through an activated carbon adsorber. A chamber identical in construction was used to expose control mice to filtered air.

The test atmospheres were generated by passing nitrogen carrier gas through a thermally controlled vapor contact zone. The vapor-saturated carrier streams were mixed with dilution air before entering the exposure compartment. For liquid test-phase articles, vapors were transpired from the reservoir of a fritted-inlet gas bubbler which was filled with the test compound. Generation of phenol vapor was accomplished by passing the carrier gas through a glass U-tube loosely packed with crystalline phenol suspended on quartz wool.

The test atmosphere was monitored with a Hewlett-Packard 5840A gas chromatograph equipped with flame ionization, nitrogen-phosphorous, and electron capture detectors as well as an automatic gas sampling valve. The instrument was calibrated using standards prepared by diluting the test compound in an appropriate organic solvent which eluted from the column sufficiently after the test article so as not to interfere with the sample peak integration.

Exposure concentrations were calculated as means and associated standard deviations from 12 time-weighted concentration determinations for single 3-hr exposures, and on the basis of the daily means for multiple exposures. In each case the last decimal of the reported actual concentrations (Table 1) reflects the precision of the analytical method and accuracy of the analysis.

Infectious aerosol challenge. The streptococcus infectivity model was used to determine the effect of the inhaled organic vapors on susceptibility to respiratory infection (Aranyi *et al.*, 1983, 1985; Ehrlich, 1980; Gardner, 1982). Mice exposed to the vapors or filtered air were simultaneously challenged with an aerosol of viable *Streptococcus zooepidemicus* (Group C). Ensuing deaths were recorded daily over a 14-day observation period.

Pulmonary bactericidal activity. ³⁵S-Klebsiella pneumonia (noncapsulated) disseminated with a Retec X-70 disposable nebulizer were used for determination of the *in vivo* bactericidal activity of alveolar macrophages (Ar-

anyi *et al.*, 1983, 1985). Bacterial clearance was determined in the lungs of individual animals from both treated and control mice that simultaneously inhaled aerosols of the viable radiolabeled bacteria. The ratio of the viable bacterial counts to the radioactive counts in each animal's lungs provides an estimate of the rate at which bacteria are destroyed 3 hr after infection.

Experimental design and statistical methods. Groups of mice received a single 3-hr inhalation exposure to one of a series of selected organic compounds at threshold limit value concentrations (Am. Conf. Govt. Indust. Hygienists, 1983) or to filtered air. For the streptococcus infectivity studies, a minimum of five replicate infectious aerosol challenges were conducted with groups of approximately 30 mice. The pulmonary bactericidal activity assay was performed with 17 to 24 mice per treatment group. If no effects on mortality were found after a single exposure, a 5-day exposure study (3 hr/day, 5 days/week) was conducted at the same concentration. If still no effects were seen, the studies with this compound were terminated. When significant effects were found in single exposures at the TLV level or above (only acetaldehyde was tested above its TLV), the exposure concentration was reduced stepwise until a no-measurable-effect level was reached for a single exposure. A multiple exposure was then conducted at this concentration. Thus, although mortality from streptococcus aerosol challenge as well as pulmonary bactericidal activity were performed after all vapor exposures, the results of the mortality studies were used as the criteria for selection of the next lower exposure level.

Mixed-model ANOVA was used to analyze pulmonary bactericidal activity (continuous response data) transformed to logarithmic values (Winer, 1971). Mortality for the single exposures was analyzed with χ^2 statistic. The log-linear model, an analog of the ANOVA that is appropriate for discrete or qualitative response data, was used for evaluating mortality data for the studies when more than one concentration was used (Haberman, 1979). Adjustments for between replication differences were made before estimating the effects of the various exposure concentrations and frequencies. After defining overall effects in either the ANOVA or the log linear model, various contrasts were used to elucidate patterns of significant effects.

RESULTS

The target and the experimentally determined actual exposure concentrations for the various compounds tested are listed in Table 1. In each case the mean concentrations were within 10% of the target values. For convenience and clarity, the target levels are the concentrations used in the text and other ta-

⁶ Fisher Scientific Co., Pittsburgh, Pa.

TABLE I
LIST OF COMPOUNDS WITH TARGET AND EXPERIMENTALLY DETERMINED CONCENTRATION
LEVELS USED IN THE STUDY

Compound name	Formula	Target	Concentration (ppm)	
			Actual ^a	
			1 Exposure	5 Exposures
Acetaldehyde	CH ₃ CHO	200 ^b	204 ± 28	180 ± 35
Acrolein	CH ₂ =CHCHO	0.10 ^c	0.09 ± 0.01	0.10 ± 0.02
Propylene oxide	CH ₃ CHOCH ₂	20.0 ^c	19.5 ± 4.3	
Chloroform	CHCl ₃	10.0 ^c	10.6 ± 1.8	10.6 ± 2.1
Methyl chloroform	CH ₃ CCl ₃	350 ^c	359 ± 53	350 ± 96
Carbon tetrachloride	CCl ₄	5.0 ^c	4.8 ± 0.7	5.0 ± 0.4
Allyl chloride	CH ₂ =CHCH ₂ Cl	1.0 ^c	1.1 ± 0.2	1.3 ± 0.3
Benzene	C ₆ H ₆	10.0 ^c	10.6 ± 2.0	10.2 ± 1.7
Phenol	C ₆ H ₅ OH	5.0 ^c	5.6 ± 0.5	5.4 ± 0.3
Monochlorobenzene	C ₆ H ₅ Cl	75 ^c	76 ± 16	71 ± 19
Benzyl chloride	C ₆ H ₅ CH ₂ Cl	1.0 ^c	1.0 ± 0.1	1.0 ± 0.1
Methylene chloride	CH ₂ Cl ₂	100 ^c	95 ± 7	
		50	52 ± 3	51 ± 5
Perchloroethylene	Cl ₂ C=CCl ₂	50 ^c	48 ± 9	
		25.0	25.6 ± 1.6	24.7 ± 7.0
Ethylene trichloride	ClCH=CCl ₂	50 ^c	48 ± 4	
		25.0	25.6 ± 4.3	
		10.0	10.6 ± 1.7	
		5.0	5.2 ± 0.8	
		2.5	2.6 ± 0.2	2.6 ± 0.3

^a Means ± SD were calculated from 12 time-weighted concentration determinations for single 3-hr exposure periods. Multiple exposure concentrations were calculated on the basis of the daily means.

^b 2X TLV level.

^c TLV level from "Threshold limit values for chemical substances in the work environment adopted by ACGIH for 1983-84".

bles. The results summarized in Table 2 show 11 compounds that did not produce significant changes in mortality of mice from streptococcal challenge after single 3-hr exposures. Only two of these agents, chloroform and allyl chloride, showed significantly increased mortality from the infection and only after five daily 3-hr exposures. Changes in pulmonary bactericidal activity were significant after single, and/or multiple exposure to some of the compounds, however, the patterns of the effects were not consistent with those shown in the mortality studies.

When mice were exposed to 100 ppm of methylene chloride and 50 ppm of perchloroethylene, significant increases in mortality

from streptococcal aerosol challenge and corresponding decreases in pulmonary bactericidal activity to inhaled *K. pneumoniae* were found after single 3-hr exposures (Table 3). Decreasing the exposure concentrations by 50% resulted in no statistically significant effects for both experimental parameters after single as well as five exposures.

Results of the exposures to various concentrations of ethylene trichloride showed a significant ($p < 0.0001$) treatment by concentration interaction for mortality, with the magnitude of the effect increasing with concentration. A significant ($p < 0.0001$) treatment by concentration interaction for bactericidal activity was also found. Exposure fre-

TABLE 2

EFFECT OF EXPOSURES TO TLV^a LEVELS OF ORGANIC VAPOR-PHASE COMPOUNDS ON MORTALITY FROM STREPTOCOCCAL PNEUMONIA OR PULMONARY BACTERICIDAL ACTIVITY TO INHALED ³⁵S-*K. pneumoniae*

Exposure			Mortality				Bactericidal activity				
Compound	PPM	Number of days (3 hr/day)	Number of replicate challenges	D/T ^b	%	Difference exp-cont	% Bacteria killed in 3 hr		Difference exp-cont	N ^c	
							Mean	SE			
Acetaldehyde	0	1	5	34/191	17.8		79.9	2.0		24	
	200 ^d			35/193	18.1	0.3	86.2*	1.6	6.3*	23	
	0	5	5	30/140	21.4		73.2	2.0		17	
	200 ^d			22/141	15.6	-5.8	62.0	3.7	-11.2*	18	
Acrolein	0	1	5	18/148	12.2		82.3	1.8		24	
	0.1			20/148	13.5	1.3	82.6	1.7	0.3	24	
	0	5	5	6/140	4.3		84.3	1.6		18	
	0.1			11/140	7.9	3.6	76.6	2.9	-7.7**	18	
Propylene oxide	0	1	5	16/135	11.8		80.0	1.9		23	
	20			25/135	18.5	6.7	76.3	2.6	-3.7	21	
	0	5	6	21/162	13.0		Not done				
	20			27/162	16.7	3.7					
Chloroform	0	1	5	8/140	5.7		83.4	1.9		18	
	10			9/140	6.7	1.0	82.9	1.4	-0.5	17	
	0	5	4	34/112	30.4		84.1	1.3		17	
	10			49/112	43.7	13.3*	84.8	0.8	0.7	18	
Methylchloroform	0	1	5	31/140	22.1		81.1	1.5		18	
	350			34/140	24.3	2.2	85.2	1.1	4.1*	16	
	0	5	5	9/140	6.4		92.6	1.0		17	
	350			10/140	7.1	0.7	91.0	1.0	-1.6	17	
Carbon tetrachloride	0	1	4	9/112	8.0		87.4	1.1		36	
	5			9/112	8.0	0.0	89.3	1.1	1.9	36	
	0	5	5	11/140	7.9		83.5	0.9		18	
	5			12/139	8.6	0.7	89.4	0.9	5.9	18	
Allyl chloride	0	1	10	21/270	7.8		71.6	2.0		40	
	1			34/270	12.6	4.8	84.0	1.4	12.4***	44	
	0	5	5	11/134	8.2		89.9	0.8		23	
	1			23/134	17.2	9.0*	94.5	1.0	4.6***	23	
Benzene	0	1	5	20/135	14.8		85.6	1.1		41	
	10			21/135	15.6	0.8	88.8	0.8	3.2*	39	
	0	5	5	18/135	13.3		85.6	2.2		23	
	10			20/135	14.8	1.5	71.9	3.0	-13.7***	23	

TABLE 2—Continued

Exposure			Mortality				Bactericidal activity			
Compound	PPM	Number of days (3 hr/day)	Number of replicate challenges	D/T ^b	%	Difference exp-cont	% Bacteria killed in 3 hr		Difference exp-cont	N ^c
							Mean	SE		
Phenol	0	1	5	15/138	10.9		73.3	2.3		18
	5			11/140	7.8	-3.1	77.1	2.4	3.8	18
	0	5	5	8/140	5.7		84.7	1.7		18
	5			10/140	7.1	1.4	87.3	1.0	2.6	17
Monochlorobenzene	0	1	5	25/140	17.9		88.4	1.0		18
	75			15/140	10.7	-7.2	90.1	0.9	1.7	18
	0	5	5	21/135	15.6		94.2	0.4		23
	75			21/135	15.6	0.0	91.8	0.7	-2.4	23
Benzyl chloride	0	1	10	40/270	14.8		82.4	1.7		44
	1			45/270	16.7	1.9	89.9	1.3	7.5**	46
	0	5	5	16/135	14.1		94.7	0.5		22
	1			14/135	10.4	-3.7	94.4	0.5	0.3	22

Note. Significantly different from control: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

^a TLV level from "threshold limit values for chemical substances in the work environment adopted by ACGIH for 1983-84."

^b Number dead/number of mice tested.

^c Number of mice.

^d Two times TLV level.

quency was not a significant factor for either parameter. Single 3-hr inhalation exposures to ethylene trichloride at 50, 25, and 10 ppm resulted in significant increases in mortality from streptococcus aerosol infection, whereas the increases after single exposures to 5 and 2.5 ppm, or five exposures to 2.5 ppm were not significant. Pulmonary bactericidal activity was significantly decreased after 3-hr inhalation of 10 ppm of ethylene trichloride, but single 3-hr exposures to 5 and 2.5 ppm caused significant increases. None of the other exposure conditions produced significant effects (Table 4).

DISCUSSION

The compounds examined in this study represent a wide variety of chemical structure,

and type and extent of usage and have been evaluated for toxicity by the American Conference of Governmental Industrial Hygienists (1980). Generally, most of the chemicals cause several types of effects including depression of the central nervous system and liver and/or kidney toxicity. A few are suspect carcinogens. Acetaldehyde and acrolein are the only chemicals examined for which TLVs have been set to protect workers from respiratory tract effects (irritation, pulmonary edema). From the published literature, it appears that only acrolein has been previously examined for effects on pulmonary host defenses (Astry and Jakab, 1983; Jakab, 1977).

The testing strategy used in these studies was based on the high degree of sensitivity of the streptococcus aerosol infectivity model in determining the net impact on several mech-

TABLE 3
EFFECT OF EXPOSURES TO METHYLENE CHLORIDE OR PERCHLOROETHYLENE ON MORTALITY FROM STREPTOCOCCAL PNEUMONIA OR PULMONARY BACTERICIDAL ACTIVITY TO INHALED *S. K. pneumoniae*

Exposure			Mortality				Bactericidal activity			
Compound	PPM	Number of days (3 hr/day)	Number of replicate challenges	D/T ^a		Difference exp-cont	% Bacteria killed in 3 hr		Difference exp-cont	N ^b
					%		Mean	SE		
Methylene chloride	0	1	5	9/140	6.4		91.6	1.1		18
	100 ^c			26/140	18.6	12.2**	79.6	2.6	-12.0***	18
	0	1	5	34/140	24.3		85.1	1.4		18
	50			39/140	27.8	3.5	86.4	1.3	1.3	18
	0	5	5	24/140	17.4		83.9	0.9		18
	50			26/140	18.6	1.2	81.3	1.6	-2.6	18
Perchloroethylene	0	1	5	21/140	15.0		80.5	1.8		18
	50 ^c			44/140	31.4	16.4**	73.9	2.3	-6.6*	18
	0	1	5	64/140	45.7		87.7	1.2		18
	25			52/140	37.1	-8.6	84.7	1.2	-3.0	18
	0	5	5	16/140	11.4		80.5	1.8		18
	25			17/140	12.1	0.7	83.2	1.8	2.7	18

Note: Significantly different from control: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

^a Number dead/number of mice tested.

^b Number of mice.

^c TLV level from "threshold limit values for chemical substances in the work environment adopted by ACGIH for 1983-84."

anisms of host defenses after exposure to inhaled toxicants. The pulmonary bactericidal activity assay was included in the studies to examine the effect of the exposures on alveolar macrophage function since they maintain the sterility of the deep lung. The 3-hr daily exposure periods were selected because an extensive data base is available for these two assays under such conditions, thereby facilitating broader potency comparisons.

These studies have demonstrated that out of 14 organic vapor-phase compounds examined, 3 (methylene chloride, perchloroethylene, and ethylene trichloride) produced significantly increased susceptibility to experimentally induced respiratory infection in mice after a single 3-hr exposure to their TLV levels. For ethylene trichloride, the no-measurable effect level for a single 3-hr exposure

was attained only after reducing the TLV concentrations by one order of magnitude.

A single 3-hr exposure to 0.1 ppm acrolein caused no significant effects on streptococcal-induced mortality or bactericidal activity, but 5 days of exposure reduced bactericidal activity. This is consistent with the research of Astry and Jakab (1983) who showed that an 8 hr exposure to ≥ 3 ppm acrolein decreased bactericidal activity in an assay similar to ours but with a different species of bacteria, while 0.5 ppm caused no effects. Jakab (1977) also found that 24 hr, but not 4 hr of exposure, to 1-2 ppm acrolein were required to reduce bactericidal activity.

For the majority of inhaled chemicals evaluated with the streptococcus aerosol infectivity model and by measuring bacterial inactivation in the lungs, when mortality following strep-

TABLE 4

EFFECT OF EXPOSURES TO ETHYLENE TRICHLORIDE ON MORTALITY FROM STREPTOCOCCAL PNEUMONIA OR PULMONARY BACTERICIDAL ACTIVITY TO INHALED ³⁵S-K. pneumoniae

Exposure		Mortality				Bactericidal activity			
PPM	Number of days (3 hr/day)	Number of replicate challenges	D/T ^a	%	Difference exp-cont	% Bacteria killed in 3 hr		Difference exp-cont	N ^b
						Mean	SE		
0	1	10	37/275	13.4		84.7	1.0		41
50 ^c			160/275	58.1	44.7***	83.7	1.2	-1.0	41
0	1	10	72/275	25.2		84.9	1.2		39
25			163/275	54.5	29.3*	83.6	1.3	-1.3	41
0	1	10	37/280	13.2		85.8	0.8		72
10			72/280	25.7	12.5*	79.5	1.6	-6.3***	72
0	1	10	36/275	13.2		67.9	1.6		37
5			57/275	20.8	7.6	74.7	1.1	6.8*	38
0	1	5	8/140	5.7		86.9	1.2		40
2.5			11/140	7.9	2.2	91.0	0.7	4.1**	39
0	5	5	16/140	11.4		90.7	1.1		18
2.5			27/140	19.5	7.9	91.0	1.0	0.3	18

Note. Significantly different from control: **p* < 0.05, ***p* < 0.01, ****p* < 0.001.^a Number dead/number of mice tested.^b Number of mice.^c TLV level from "threshold limit values for chemical substances in the work environment adopted by ACGIH for 1983-84."

tococcal infection increases, pulmonary bactericidal activity decreases (Goldstein *et al.*, 1974; Ehrlich *et al.*, 1977; Aranyi *et al.*, 1981; Sherwood *et al.*, 1981; Gardner, 1984; Aranyi *et al.*, 1985). Such correlated responses imply that the infectivity model is primarily dependent on the function of alveolar macrophages. However, studies in our laboratory have also shown that inhalation of certain test atmospheres produced increased mortality in the presence of increased pulmonary bactericidal activity (Aranyi *et al.*, 1983). In other cases, especially close to the no-measurable effect levels, unchanged mortality was associated with increased pulmonary bactericidal activity (Aranyi *et al.*, 1981). The single exposures to acetaldehyde, allyl chloride, benzene, and benzyl chloride belong into this latter category. For ethylene trichloride, both increases and

decreases in bactericidal activity were observed, depending on concentration and number of exposures. The mechanisms for this differential response are not clear. It may be speculated that whereas exposures at the lower concentrations had no effect on mortality, bactericidal activity increased, perhaps due to a pollutant-induced recruitment of unexposed alveolar macrophages. At the higher concentrations, however, increases in mortality but no significant changes in bactericidal activity occurred, suggesting that the mechanisms of toxicity involved obscured the stimulative effects on macrophages seen at the lower concentrations. Thus increased mortality in the absence of suppression in pulmonary bactericidal activity indicates that, in certain inhalation exposure systems, alveolar macrophages are either not adversely affected in

phagocytosis and killing, or are able to compensate to maintain bactericidal activity at or above normal levels. Although the actual mechanisms involved can only be evaluated with further research, these data illustrate the importance of exploring a rather full range in concentration-response studies.

Inhaled toxicants can prolong bacterial viability in the lung and thus enhance severeness of disease. The capability of the host to protect itself depends on phagocytic and bactericidal activity of alveolar macrophages, as well as other complex interdependent pulmonary and extrapulmonary defense mechanisms (Green *et al.*, 1977). These studies provide important information on the toxicity of inhaled test compounds which alter susceptibility to respiratory infection in the host. This fast and cost-effective testing method can identify potentially hazardous agents that should be further investigated.

ACKNOWLEDGMENTS

These studies were supported by funds provided by the U.S. Environmental Protection Agency under Cooperative Agreement CR807434. This report has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement of recommendation for use. The authors are indebted to Dr. Thomas Williams (Northrop Services, Inc.) for his technical advice on the vapor generation system and to Dr. Robert Gibbons for statistical analysis of the data. They also thank Mr. William Mega, for his excellent technical assistance.

REFERENCES

- American Conference of Governmental Industrial Hygienists (1980). *Documentation of the Threshold Limit Values*, 4th ed. Cincinnati, Ohio.
- American Conference of Governmental Industrial Hygienists (1983). *TLVs. Threshold Limit Values for Chemical Substances in Workroom Environment with Intended Changes for 1983-1984*. ISBN: 0-936712-45-7.
- ARANYI, C., BRADOF, J. N., GARDNER, D. E., AND LEW-TAS-HUISINGH, J. (1981). *In vitro* and *in vivo* evaluation of potential toxicity of industrial particles. In *Short-term Bioassays in the Analysis of Complex Environmental Mixtures II* (M. D. Waters, S. S. Sandhu, J. Lewtas-Huisingh, L. Claxton, and S. Nesnow, eds.), pp. 431-443. Plenum, New York.
- ARANYI, C., BRADOF, J. N., O'SHEA, W. J., GRAHAM, J. A., AND MILLER, F. J. (1985). Effects of arsenic trioxide inhalation exposure on pulmonary antibacterial defenses in mice. *J. Toxicol. Environ. Health* **15**, 163-172.
- ARANYI, C., VANA, S. C., THOMAS, P. T., BRADOF, J. N., FENTERS, J. D., GRAHAM, J. A., AND MILLER, F. J. (1983). Effects of subchronic exposure to a mixture of O₃, SO₂, and (NH₄)₂SO₄ on host defenses of mice. *J. Toxicol. Environ. Health* **12**, 55-71.
- ASTRY, C. L., AND JAKAB, G. J. (1983). The effects of acrolein exposure on pulmonary antibacterial defenses. *Toxicol. Appl. Pharmacol.* **67**, 49-54.
- EHRlich, R. (1980). Interaction between environmental pollutants and respiratory infections. *Environ. Health Perspect.* **35**, 89-100.
- EHRlich, R., FINDLAY, J. C., FENTERS, J. D., AND GARDNER, D. E. (1977). Health effects of short term inhalation of nitrogen dioxide and ozone mixtures. *Environ. Res.* **14**, 223-231.
- GARDNER, D. E. (1984). Alteration in macrophage function by environmental chemicals. *Environ. Health Perspect.* **55**, 343-358.
- GARDNER, D. E. (1982). Effects of gases and airborne particles on lung infections. In *Air Pollution—Physiological Effects* (J. J. McGrath and C. D. Barnes, eds.), pp. 47-79. Academic Press, Orlando, Fla.
- GOLDSTEIN, E., WARSHAUER, D., LIPPERT, W., AND TARKINGTON, B. (1974). Ozone and nitrogen dioxide exposure. *Arch. Environ. Health* **28**, 85-90.
- GREEN, G. M., JAKAB, G. J., LOW, R. B., AND DAVIS, G. S. (1977). Defense mechanisms of the respiratory membrane. *Amer. Rev. Resp. Dis.* **115**, 479-514.
- HABERMAN, S. J. (1979). *Analysis of Qualitative Data*. Academic Press, Orlando, Fla.
- JAKAB, G. J. (1977). Adverse effect of a cigarette smoke component acrolein on pulmonary antibacterial defenses and on viral-bacterial interactions in the lung. *Amer. Rev. Resp. Dis.* **115**, 33-38.
- SHERWOOD, R. L., TARKINGTON, B., LIPPERT, W. E., AND GOLDSTEIN, E. (1981). Effect of ferrous sulfate aerosols and nitrogen dioxide on murine pulmonary defense. *Arch. Environ. Health* **36**, 130-135.
- U.S. International Trade Commission (1982). *United States Production and Sales, 1981*. U.S. Government Printing Office.
- WINER, B. J. (1971). *Statistical Principles in Experimental Design*. McGraw-Hill, New York.

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

This article documents research that was conducted under the auspices of the U.S. Environmental Protection Agency (USEPA). As an arm of the government, the USEPA has a paid up, non-exclusive, irrevocable, worldwide license to reproduce, prepare derivative works, and distribute to the public all of the material contained herein, and to grant others acting on its behalf the right to do so. The USEPA grants to the National Technical Information Service, Department of Commerce, the right to act as its agent in the distribution of this material.